

REMARKS

Claims 19 and 21-29 are pending. Claims 1-18 and 30-41 have been cancelled without prejudice as relating to a non-elected invention. Claim 20 has also been cancelled without prejudice. Claim 19 has been currently amended to specify that cells of the pancreatic islet are cultured under conditions wherein cultured cells comprise nestin-positive cells which have migrated from the islet. New claim 42, which depends from claim 19, adds the additional limitation "wherein the migrated cells form a monolayer". Claim 22 has been currently amended to specify that the nestin-positive pancreatic stem cell is an "isolated" cell. New claim 43 has been added to recite the process steps of one embodiment using concanavalin A. New claim 44 to an isolated nestin positive pancreatic stem cell isolated by the methods of the invention has been added. Support for these amendments and new claims 42-44 are found throughout the specification. No new matter is added by this amendment.

Applicants wish to thank Examiner Wehbe for the courtesy extended to their attorneys during a telephonic interview on February 4, 2004. Applicants believe the interview was helpful and productive, the essence of which is summarized below. As per our telephone conversation with the Examiner, Applicants believe that the amendments to claim 19 will obviate the rejection of claims 19-21 under 35 U.S.C. §112, second paragraph.

Applicants have filed separately a petition to accept unintentionally delayed claim for priority under 35 U.S.C. 119(e) to U.S. Provisional Application No. 60/169,082, filed December 6, 1999 and U.S. Provisional Application 60/215,109, filed June 28, 2000, in addition to U.S. Provisional Application 60/238,880, filed October 6, 2000, to which priority has been claimed and acknowledged.

In view of the above, applicants submit that the effective filing date of the instant application is December 6, 1999.

Rejection of Claims 19-21 under 35 U.S.C. §112, Second Paragraph

Claims 19-21 remain rejected under 35 U.S.C. §112, second paragraph, for allegedly being incomplete for omitting essential steps. During the above-referenced interview, Examiner

Wehbe characterized the rejection as requiring an intervening step of “identifying” a nestin positive clone prior to the selection step. Applicants appreciate this clarification and have amended claim 19 accordingly. Claim 20 has been cancelled. In view of this amendment, Applicants respectfully request reconsideration and withdrawal of the rejections.

Support for the amendment to claim 19 and new claim 42 is found throughout the specification and in particular at page 26, lines 4-6 wherein the specification states that “[t]he inventors discovered that nestin expressing cells grow out of cultured islets and can be observed growing around the islets as early as about four days in culture”; at page 39, lines 22-24 wherein the specification states that “[t]he islets adhered to the surface of the plates, and cells grew out and away from the islets in a monolayer. . . cells that form a monolayer were nestin-positive by immunostaining with a rabbit anti-rat nestin antiserum”; at page 48, lines 25-28, wherein the specification states that “[t]he islets attached to the plates and cells slowly grew out of the islet as a monolayer...[t]he outgrowing monolayer of cells were phenotypically homogenous...and expressed nestin”; at page 15, lines 24-25, wherein Figure 3 is described as depicting “nestin-positive cells that have proliferated out from a cultured rat islet; in Figure 3; and at page 40, lines 2-4 wherein the specification states “[a]fter two weeks of culture, several (3-5) of the nestin-positive monolayer cells were removed by picking with a capillary tube (cylinder cloning).”

Applicants wish to clarify that the method of the present invention, as currently claimed, includes the step of culturing the cells from the pancreatic islet under conditions wherein nestin-positive cells are identified by the migration of the nestin-positive cells from the islet (claim 19) or by the formation of a monolayer following migration of the nestin-positive cells from the islet (claim 42). In carrying out the claimed invention, the cells are cultured under conditions that allow them to be differentiated and separated from other cell types by their migration from the islet (claim 19) or by the formation of a monolayer after migration from the islet (claim 42). Although the specification teaches a step wherein nestin-positive cells are separated from other cells by culturing the cells on concanavalin A coated plates, there are both pre and post-filing publications, as well as post-filing data from the inventor’s laboratory that teach methods of isolating a stem cell from a pancreatic islet of Langerhans that do not require culturing on concanavalin A.

For example, attached hereto is a post-filing publication (Cattaneo et al., 1990, *Nature*, 347:762-765, Exhibit A) that describes an alternate method of isolating a nestin-positive cell, specifically a nestin-positive neuronal stem cell. According to this method, “embryonic rat striatum primordia were dissected . . . and cultured in serum-free medium. Immediately after plating, more than 95% of rat striatal cells dissociated from embryonic day 13.5-14.5 expressed nestin, specifically found in neuroepithelial stem cells.”

Also attached hereto is a post-filing publication from the inventor’s laboratory (Abraham et al., 2002, *Endocrinology*, 143:3152, Exhibit B) wherein nestin-positive islet-derived progenitor cells are identified as follows: “islets were washed and cultured in RPMI 1640 medium containing serum, 11.1 mM glucose, antibiotics, sodium pyruvate, β -mercaptoethanol, and growth factors. Within several days, nestin-positive progenitor cells grew out from islets. These cells were cloned and expanded in medium containing 20 ng/ml each of bFGF and EGF.”

Applicants have also attached a Rule 1.132 declaration of Dr. Habener stating that nestin-positive cells can be identified by digesting islets with trypsin to prepare single cell suspensions of human pancreatic islet preparations and growing the resulting cells in an appropriate medium, for example RPMI 1640 (11 mmol/l glucose) with 10 mmol/l Hepes buffer, 1 mmol/l sodium pyruvate, 10% fetal bovine serum, 25 ng/ml EGF, 20 ng/ml bFGF and 1X penicillin/streptomycin. The resulting expansion cultures of progenitor cells contain two major populations of cells that are phenotypically distinct cell types; those that express nestin and vimentin and those that express epithelial markers cytokeratin 19 and E-cadherin, as detected by immunofluorescence (see attached figure, Exhibit C).

The two major populations of cells are easily separated based on differences in their morphologies. The nestin/vimentin positive spindle shaped fibroblast-like cells are markedly different from that of the E-cadherin/CK19 positive, flat, cuboidal epithelial-like cells that are in patches. Under regular or phase contrast light microscopy, using low power, nestin/vimentin positive cells that are clearly separated from the E-cadherin/CK19 cells which grow in distinct patches, are selected.

Based on the forgoing, one skilled in the art will appreciate that a variety of separation strategies based on immunophenotyping methodologies such as surface coated antibody panning, fluorescent antibody tagging for physical isolation, flow cytometric sorting, immunomagnetic bead and particle selection and counterselection will be useful in carrying out the present invention. As shown herein, a number of selection criteria can be employed to allow the isolation of a distinct populations of nestin+/vimentin+/cytokeratin 19-/E-cadherin- cells . It is also appreciated by one skilled in the art that other markers known in the field, using similar separation strategies, can be employed to isolate distinct populations of nestin+ cells.

Support for such methods is also disclosed in the instant specification which states at page 18, lines 16-19 that “[s]tem cells according to the invention can be identified by their expression of nestin by, for example, FACS. immunocytochemical staining, RT-PCR. Southern, Northern and Western blot analysis, and other such techniques of cellular identification as known to one skilled in the art.” The instant specification characterizes the markers present on the stem cells of the invention (see page 22) and also discloses antibodies to stem cell markers including nestin, vimentin and cytokeratin-19 (see page 19).

Based on the foregoing, one of skill in the art will appreciate that a variety of culture media will be useful in carrying out the present invention, including but not limited to serum-free medium, or “RPMI 1640 medium containing serum, 11.1 mM glucose, antibiotics, sodium pyruvate, β -mercaptoethanol, and growth factors”, as discussed above. As shown herein, a number of culture media exist which are useful for culturing and identifying a nestin-positive cell.

In view of the above, Applicants submit that claims 19-29 satisfy the requirements of 35 U.S.C. § 112, second paragraphs, and request reconsideration and withdrawal of the rejection.

Rejection of Claims 22-23 Under 35 U.S.C.  102(a)

Claims 22-23 remain rejected under 35 U.S.C.  102(a) as allegedly being anticipated by Stoffers et al. (2000), *Diabetes*, Vol. 49, 741-748. Applicants respectfully traverse this rejection.

Applicants submit that the effective filing date of the instant application is December 6, 1999 (see discussion in Remarks session). In view of the above, the Stoffers et al. reference is not prior art to the present application..

In view of the above, Applicants respectfully request reconsideration and withdrawal of the 35 U.S.C.  102(a) rejection of claims 22-23 in view of Stoffers et al.

Rejection of Claims 22-23 Under 35 U.S.C.  102(b)

Claims 22-23 remain rejected under 35 U.S.C. 102(b) as allegedly being anticipated by Xu et al. (1999) *Diabetes*, Vol. 48, 2270-2276.

The Examiner states that “[t]he rejection as written is based on the fact that Xu et al. teaches the exact same method steps as applicant’s claims as written. The only method step in the claim is ‘treating a nestin-positive pancreatic stem cell with an agent selected from a group which includes IDX-1 . . . the pancreas does in fact contain nestin-positive pancreatic stem cells. Xu et al. further teaches that the administration of exendin-4 *in vivo* in rats results in the differentiation of new  -cells from pancreatic progenitor cells present in the pancreas . . . Thus, since Xu et al. teaches that the pancreatic progenitor cells present in the pancreas are responding to the exendin-4 treatment, the skilled artisan would not doubt that cells present in the pancreas, which include nestin-positive cells, are exposed to exendin-4 following intraperitoneal administration.” Applicants respectfully traverse this rejection.

Claim 22 and dependent claim 23 has been amended to recite “a method of inducing the differentiation of an **isolated nestin-positive pancreatic stem cell** into a pancreatic progenitor cell, comprising the step of: treating a nestin-positive pancreatic stem cell with an agent selected from the group consisting of EGF, bFGF-2, high glucose, KGF, HGF/SF, IDX-1, a nucleic acid molecule encoding IDX-1, GLP-1, exendin-4, betacellulin, activin A, TGF- , and combinations

thereof, whereby the stem cell subsequently differentiates into a pancreatic progenitor cell (emphasis added).”

Support for this amendment is found throughout the specification and in particular at page 23, line 19- page 26, line 15, in the section entitled, “Isolated Stem Cells from Pancreatic Islets and Their Uses”, and in Example 2, wherein isolated stem cells are induced to differentiate to form islets.

As stated in the instant specification at p. 10, lines 9-12, a “pancreatic” stem cell means “a stem cell that has been **isolated** from pancreatic tissue and/or a cell that has all of the characteristics of: nestin -positive staining, nestin gene expression, cytokeratin-19 negative staining, long-term proliferation in culture, and the ability to differentiate into pseudo-islets in culture” (emphasis added).

“Isolated” is defined in the specification at page 11, lines 3-15 as “the process of removing a stem cell from a tissue sample and separating away other cells which are not stem cells of the tissue. An isolated stem cell will be generally free from contamination by other cell types and will generally have the capability of propagation and differentiation to produce mature cells of the tissue from which it was isolated.”

Applicants submit that for a determination of anticipation to be proper, the prior art reference must disclose each and every limitation of the claim. *Atlas Powder Company et al. v. IRECO, Incorporated et al.*, 190 F.3d 1342, 1347 (Fed. Cir. 1999).

The Xu et al. reference teaches administration of exendin-4 to **rats** for 10 days, wherein administration is via a daily intraperitoneal injection of 1nmol/kg and wherein the rats have undergone either a sham surgical procedure or a 90-95% pancreatectomy. The Xu et al. reference does not teach “a method of inducing the differentiation of an **isolated** nestin-positive pancreatic stem cell into a pancreatic progenitor cell (emphasis added)” as required by claims 22 and 23 of the instant application. In view of the above, the Xu et al. reference does not disclose each and every limitation of claims 22 and 23 of the instant application.

In view of all of the above, Applicants respectfully request reconsideration and withdrawal of the 35 U.S.C. 102(b) rejection of claims 22 and 23 in view of Xu et al.

Rejection of Claims 24-29 Under 25 U.S.C. §102(e)

Claims 24-29 are rejected under 35 U.S.C. 102(e) as allegedly being anticipated by U.S. Patent No. 6,436,704 (8/20/02). Applicants respectfully traverse.

Applicants submit that the effective filing date of the instant application is December 6, 1999 (see discussion in Remarks session). In view of the above, the U.S. 6,436,704 patent (8/20/02) is not prior art to the present invention.

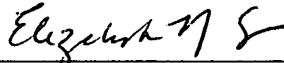
In view of the above, Applicants respectfully request reconsideration and withdrawal of the 35 U.S.C. §102(e) rejection of claims 24-29 in view of U.S. 6,436,704.

CONCLUSION

Applicant submits that all claims are allowable as written and respectfully request early favorable action by the Examiner.

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Respectfully submitted,



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